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Stephen Johnson, Administrator
U.S. Environmental Protection Agency
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1200 Pennsylvania Ave. NW
Washington, DC 20460

Comments on the HPV test plan for 1,6-bis(dibutylethylammonium)hexane hydroxide (BQAOH) and 1,6-bis(dibutylethylammonium)hexane ethysulfate (BQAES)

Dear Administrator Johnson:

Solutia has submitted an HPV test plan for BQAOH (CAS no. 111960-92-0) and BQAES (CAS no. 68052-49-3). The following comments on this test plan are submitted on behalf of People for the Ethical Treatment of Animals, the Physicians Committee for Responsible Medicine, the Humane Society of the USA, the Doris Day Animal League, and Earth Island Institute. These health, animal-protection and environmental organizations have a combined membership of more than ten million Americans.

BQAOH is used in the manufacture of nylon-6,6 and BQAES is an intermediate in the manufacture of BQAOH. These compounds are thus produced and consumed on site. We commend Solutia for its decision not to carry out additional mammalian studies on BQAOH or BQAES, which the company states are "known to be corrosive to the skin and eyes and potentially lethal upon dermal exposure." On occasions in the past, EPA has encouraged companies to circumvent the testing of corrosive chemicals on animals. In one example, EPA states that "based on the strong acidic and corrosive nature of the substance, EPA believes that the sponsor needs to consider whether the proposed testing would yield meaningful results." (See http://www.epa.gov/chemrtk/bnzslfad/c14743tc.htm and http://www.epa.gov/chemrtk/hydrbnsa/c14744tc.htm)

In addition to the fact that these compounds are known to have high acute toxicity and corrosivity, it is clear that the results of repeated-dose studies would not lead to changes in handling procedures (test plan, p. 14). Because these chemicals are produced and consumed on site and pose a known extreme hazard, a high level of engineering controls and personal protection are already in place.

Solutia's decision represents an appropriate application of the following ruling from the EPA:

> In analyzing the adequacy of existing data, participants shall conduct a thoughtful, qualitative analysis rather than use a rote checklist approach. Participants may conclude that there is sufficient data, given the totality of what is known about a chemical, including human experience, that certain endpoints need not be tested (*Federal Register* 12-26-2000).

Although we are pleased to see the application of thoughtful toxicology and Solutia's consideration of animal welfare as described in the test plan (p. 14), we disagree with Solutia's

proposal to carry out an acute toxicity test on fish, which will kill approximately 60 animals per test.

Solutia has evaluated potential toxicity to aquatic life using ECOSAR, and thus predicted BQAOH and BQAES to be of low toxicity to aquatic species (test plan, p. 3). We recommend that Solutia use *in vitro* methods, instead of additional animal tests, for its assessment of fish toxicity. This is a scientifically valid analysis of ecotoxicity and sufficient for a screening level program such as HPV.

The recently validated *Dar*T Test (Nagel 2002) is a prospective replacement for *in vivo* studies. The test protocol and performance parameters are described in detail in Schulte (1994) and Nagel (1998). Briefly, the *Dar*T test uses fertilized zebrafish (*Danio rerio*) eggs as a surrogate for living fish. Because the eggs will not hatch during the test period, the *Dar*T is classified as a non-animal test. The exposure period is 48-hours, and assessed endpoints include coagulation, development of blastula, gastrulation, termination of gastrulation, development of somites, movements, extension of the tail, development of eyes, heartbeat, circulation, heart rate, pigmentation, and edema. Endpoints comparable to lethality *in vivo* include failure to complete gastrulation after 12-hours, no somites after 16-hours, no heartbeat after 48-hours, and coagulated eggs. The other endpoints provide further insight for a more detailed assessment of the effects of test substances. The reliability and relevance of the *Dar*T test have recently been confirmed through an international, multi-laboratory validation study coordinated and financed by the German Environmental Protection Agency and predictions of acute toxicity from the *Dar*T test were highly concordant with *in vivo* reference data (Schulte, 1996). This *in vitro* test has been accepted in Germany as a replacement for the use of fish in the assessment of wastewater effluent (Friccius, 1995), and has since been nominated for development into an OECD test guideline. It is clearly suitable for immediate use as a replacement to the use of fish in SIDS screening studies.

Another promising *in vitro* assay is TETRATOX. In this assay, the protozoan *Tetrahymena pyriformis* is used as a biomarker for acute lethality in fish (Schultz, 1997). The biochemistry and physiology of *T. pyriformis* have been thoroughly investigated since the 1950s and this assay has been used, in various forms, for aquatic toxicity testing since the 1970s (Sinks, 2001). In this test, a range-finding study followed by three replicate definitive tests is performed for each test substance. Each treatment replicate consists of a minimum of five different concentrations per substance tested; thus, at least 30 data points comprise each analysis. The current, standardised protocol is for a 40-hour static test, which provides for multigenerational exposure. Range-finding tests are also included to allow an accurate approximation of both the highest concentration with no observed effect on population growth and the lowest concentration with total inhibition of cell replication. Output measures from the TETRATOX assay are the 50 percent inhibitory growth concentration (IGC50, mmol/L) and the 95 percent fiducial interval. The current TETRATOX database includes more than 2,000 industrial organic chemicals, including over 800 aliphatic chemicals, 900 aromatic chemicals, 400 neutral narcotics, and 400 direct-acting electrophiles, among others (Schultz, personal communication). The TETRATOX protocol has now been standardised and has undergone a preliminary ring test (Larsen, 1997). The German EPA is currently funding a second, more elaborate ring test, with the goal of establishing an OECD test guideline. In the interim, data generated by TETRATOX demonstrate

a consistently high degree of concordance to data from *in vivo* acute studies in fish, which supports the use of this assay as a prospective replacement for toxicity studies in fish (Seward, 2001).

The ecologic significance of fish tests should also be taken into consideration. Ecotoxicity and mammalian toxicity tests have different purposes: fish tests are not intended to predict toxicity in individual fish, but to predict economic loss to commercial and "sport" fisheries, and ecological damage. The fish test therefore aims to show whether exposure to BQAOH and/or BQAES would result in large-scale fish death. However, because water pollution kills the food on which fish subsist, it can deplete fish populations with no direct fish toxicity. The toxicity of BQAOH and BQAES towards aquatic invertebrates and algae, which are the food of most fish species, is unknown, as shown by the inclusion in the test plan of tests on aquatic invertebrates and algae. Fish tests should not be carried out while other types of aquatic toxicity are uncertain.

Thank you for your attention to these comments. Please feel free to contact me at 757-622-7382, ext. 8001, or via e-mail at JessicaS@peta.org.

Sincerely,

Jessica Sandler
Federal Agency Liaison

References

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